https://doi.org/10.33472/AFJBS.6.14.2024.8632-8649

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

DESIGN,CHARACTERIZATIONAND IN VITRO EVALUATION OFNANOPARTICULATE DRUG CARRIERS

Ms. Shraddha V. Chalke[1], Dr. Ajay R. Fugate[2]*,Dr. Sujit K. Nagare[3], Mr. Adarsh U. Terse[1], Mr. Tushar S. Malandkar[1].

[1]Department of Pharmaceutics, Govindrao Nikam College of Pharmacy, Sawarde.

[2]Department of Pharmaceutics, Shivlingeshwar college of Pharmacy, Almala, Latur, Email: fugatear@gmail.com

[3]Department of Pharmaceutics, Dnyandeep college of Pharmacy, Boraj, Khed, Ratnagiri

Article Info

volume 6, Issue 14, 2024

Received: 15 June 2024

Accepted: 25 July 2024

Published: 15 Aug 2024

doi: 10.48047/AFJBS.6.14.2024.8632-8649

ABSTRACT

Mesalamine, also known as 5-aminosalicylic acid (5-ASA), is an anti-inflammatory drug used to treat inflammatory bowel disease (IBD). The primary goal of this study is to develop and evaluate a nanoparticulate drug delivery system using mesalamine incorporated into polymeric micelle-filled pulsincaps to treat IBD. The solubility comparison between the pure drug and the polymeric micelles showed that mesalamine in polymeric micelles had better solubility in both water and phosphate buffer at pH 6.8. FTIR and DSC analyses confirmed no interaction between the drug and excipients. Critical Micelle Concentration studies were conducted, and various polymeric micelle formulations containing 250 mg of the drug were prepared and evaluated for various different parameters. The optimized polymeric micelle solution was freeze-dried into a free-flowing powder and assessed for compatibility, scanning electron microscopy, and flow properties. Pulsincaps were prepared by coating the capsule body with ethyl cellulose and the cap with the enteric coating material Eudragit S100. The free-flowing polymeric micelle powder was filled into the capsule bodies, sealed with HPMC K100M. Dissolution studies showed the %CDR from the mesalamine-loaded polymeric micelle-filled pulsincaps was 91.63±0.10% after 12 hours. Stability studies of the polymeric micelles were conducted according to ICH guidelines.

Keywords: Inflammatory Bowel Disease (IBD), Polymeric Micelle, Pulsincap, Mesalamine.

INTRODUCTION

The most prevalent Inflammatory Bowel Diseases (IBDs) are Crohn's disease and ulcerative colitis. Despite their similarities, the location and pattern of inflammation differ between the two. Crohn's disease causes inflammation throughout the entire gastrointestinal system, while ulcerative colitis is limited to the alimentary canal, primarily the colon. Mesalamine, or 5-aminosalicylic acid (5-ASA), has been used as an anti-inflammatory treatment for IBD for several years. It acts partially on the intestinal and colonic mucosa and has minimal complications, making it effective for preventing exacerbations of Crohn's disease and ulcerative colitis. Mesalamine falls under class IV of the Biopharmaceutics Classification System (BCS), indicating low water solubility and low permeability, which results in low bioavailability.

Nanotechnology, focusing on systems functioning at the nanoscale, involves objects or structures with diameters between one and one hundred nanometers (nm).In pharmacology, "nano" refers to particles or structures with diameters less than 1000 nm. A major challenge in treating many diseases has been the targeted delivery of therapeutic substances

to the site of action. Traditional administration methods often face limitations in efficacy, selectivity, residence times, and biodistribution. Polymeric micelles, which are self-assembled core-shell nanostructures (10 – 200 nm) made from amphiphilic block copolymers, form in aqueous solutions when the block copolymer concentration exceeds the critical aggregation concentration (CAC) or critical micelle concentration (CMC).

Targeted drug delivery to the colon is highly desirable for treating various bowel disorders such as ulcerative colitis, Crohn's disease, amebiasis, colonic cancer, and for the systemic distribution of protein and peptide medicines. The colon-specific drug delivery system (CDDS) should protect the medication on route to the colon, preventing drug release or absorption in the stomach or small intestine, and ensuring it only occurs in the colon.

Polymeric micelles are self-build core-shell nanostructures (10 – 200 nm) made of amphiphilic block copolymers that are formed in an aqueous solution. When the concentration of the block copolymer raises above a particular concentration known as the critical aggregation concentration (CAC) or critical micelle concentration (CMC), micelles form in aqueous solutions.Pulsatile drug delivery involves the rapid and transient release of a specific amount of drug following a predetermined off-period or latency, with no drug release during the lag time. This delivery method is advantageous as it ensures the medication is released quickly and completely after the lag period. Pulsatile drug delivery systems (PDDS) have become popular due to their numerous benefits over traditional dosage form.

MATERIALS AND METHODS

Materials

Mesalamine was generously provided by Dhamtec Pharma and Consultants, India. Soluplus, Pluronic F-127, and Eudragit S100 were supplied by Evonik Pvt. Ltd. Hydroxypropyl Methyl Cellulose K100M, ethyl alcohol, acetone, and all other chemicals used in the study were procured from Loba Chemicals Pvt. Ltd. (Mumbai, India).

Methods

Pre-formulation studies

Organoleptic properties: The drug samples were evaluated for its color, odor and appearance.

Melting Point determination: Melting point of Mesalamine was determined by using Thiel's tube and DSC Analysis.

Determination of Solubility: The solubility of mesalamine in various solvents, including water, acetone, 0.1N HCL, phosphate buffer pH 6.8, and phosphate buffer pH 7.4, was determined using the shake flask method. An excess amount of mesalamine was added to each volumetric flask containing 2 ml of the selected solvent and thoroughly mixed. The flasks were then placed on a water bath shaker and agitated for 24 hours at 25°C. After this period, samples were collected, centrifuged, and filtered through a 0.22 µm membrane filter. The filtrates were analyzed using an ultraviolet (UV) spectrophotometer at 232 nm to measure the amount of dissolved drug.

Determination of λmax: A 10 mg sample of standard mesalamine was accurately weighed and transferred to a 100 ml volumetric flask. It was then dissolved and diluted to the mark with a buffer solution of pH 6.8 to create a stock solution with a concentration of 100 µg/ml. The resulting solution was properly diluted and scanned from 200-400 nm to determine its maximum absorption.

Calibration Curve of Mesalamine in different buffer solutions

Calibration Curve in 0.1 N HCL

Procedure: 10 mg of Mesalamine was accurately weighed and dissolved in 100 mL of 0.1 N HCl to prepare a stock solution with a concentration of 100 µg/mLVarious aliquots were then taken from this stock solution and diluted to 10 mL with 0.1 N HCl in a series of volumetric flasks, achieving a concentration range of 10-100 µg/mL. One of these solutions was scanned in the UV range using 0.1 N HCl as a blank, and the wavelength of maximum absorption was determined to be approximately 232 nm. The absorbance of solutions with different concentrations was measured at 232 nm using 0.1 N HCl as a blank. A calibration curve was then plotted with absorbance versus concentration.

Calibration Curve in Phosphate Buffer pH 7.4

Procedure: 10 mg of Mesalamine was precisely weighed and dissolved in 100 mL of pH 7.4 buffer to prepare a stock solution (100 µg/mL). Various aliquots were withdrawn from the stock standard and diluted to various concentrations ranging from 10 to 100 µg/mL in 10 mL volumetric flasks using pH 7.4 buffer. One of these solutions was analyzed in the UV range using pH 7.4 buffer as a blank, and the wavelength of maximum absorption was determined to be approximately 232 nm. The absorbance of solutions at different concentrations was measured at 232 nm using pH 7.4 buffer as a blank. A calibration curve was constructed by plotting absorbance against concentration.

Calibration Curve in Phosphate Buffer pH 6.8

Procedure: 10 mg of Mesalamine was precisely weighed and dissolved in 100 mL of pH 6.8 buffer to prepare a stock solution (100 µg/mL). Various aliquots were withdrawn from the stock standard and diluted in 10 mL volumetric flasks using pH 6.8 buffer to achieve concentrations ranging from 10 to 100 µg/mL. One of these solutions was scanned in the UV range using pH 6.8 buffer as a blank, and the wavelength of maximum absorption was determined to be approximately 232 nm. The absorbance of solutions at different concentrations was measured at 232 nm using pH 6.8 buffer as a blank. A calibration curve was constructed by plotting absorbance against concentration.

Drug excipient Compatibility Study

Fourier Transform Infra-Red (FTIR): To assess the compatibility of the drug in the formulations, FTIR spectra of the formulations, as well as the pure drug and other excipients, were acquired and compared using an FTIR spectrophotometer.

Differential scanning Calorimetry (DSC): Differential scanning calorimetry (DSC) is a thermal analysis technique that measures the difference in heat flow required to raise the temperature of a sample compared to a reference material, as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment.

Determination of critical micelle concentration with different ratios of polymers

The critical micelle concentration (CMC) of Pluronic F-127 and Soluplus individually and in various combinations (ratios 2:1, 3:1, 4:1, 2:2, 3:2, 4:2, 2:3, 3:3, 4:3) was determined using iodine UV-visible spectroscopy. In brief, a KI/I2 stock solution was prepared by dissolving 0.5 g I2 and 1 g potassium iodide (KI) in 50 mL distilled water. A series of aqueous micellar and mixed micellar solutions were prepared with concentrations ranging from 0.00001% to 0.1%. Each solution received a 1 mL aliquot of the KI/I2 standard and was incubated at room temperature for 12 hours before analysis. UV-visible spectrophotometry at 225 nm was employed to measure the absorbance of the polymer solutions. Experiments were conducted in triplicate, and the absorbance values were plotted against the logarithm of the concentrations to determine the CMC.

Formulation Studies

Preparation of Mesalamine loaded polymeric micelle

Mesalamine-loaded polymeric micelles were prepared using the solvent evaporation technique. Initially, Mesalamine (250 mg) was dissolved in 20 mL of acetone along with Soluplus and Pluronic F127. The acetone was then removed by suction using a rotary evaporator at 55°C. Afterward, the resulting film was soaked in 10 mL of deionized water and agitated for 30 minutes to remove non-incorporated Mesalamine. The solution containing Mesalamine-loaded polymeric micelles was obtained by passing it through a 0.45 µm membrane. Blank mixed micelles were also prepared using the same method, excluding the addition of Mesalamine.

Optimization of Mesalamine loaded Polymeric Micelle

To improve formulation preparation, 9 batches along with the blank polymeric micelle were evaluated for particle size, polydispersity index, % entrapment efficiency, and zeta potential.

Table 1: Optimization of Mesalamine loaded Polymeric Micelle

Batch Code	Drug (mg)	Soluplus (mg)	Pluronic F-127(mg)
F1	250	200	100
F2	250	300	100
F3	250	400	100
F4	250	200	200
F5	250	300	200
F6	250	400	200
F7	250	200	300
F8	250	300	300
F9	250	400	300

Characterization of Polymeric Micelle Solution

Determination of Particle size and Polydispersity Index: The particle size and polydispersity index (PDI) of both the polymeric micelle solution and the blank micelle were analyzed using photon correlation spectroscopy (PCS). This technique examines fluctuations in light scattering caused by the Brownian motion of particles. Measurements were conducted with a NanoSizer SZ-100 (Horiba NanoSize Analyzer, Japan), capable of detecting sizes ranging from 10 to 3000 nm.

Determination of Zeta potential: The zeta potential of polymeric micelles was measured using the Zetasizer HAS 3000 (Malvern Instruments Ltd., UK). Transparent, disposable zeta cells were utilized to contain the samples, and the readings were recorded. Before introducing each new sample, cuvettes were rinsed with methanol and then cleaned with the liquid under analysis.

Entrapment Efficiency: The amount of free drug in the supernatant after centrifugation can be assessed to determine the entrapment efficiency. A freshly prepared polymeric micelle solution (10 mL) was centrifuged at 1000 rpm for 10 minutes. The UV-Visible spectrophotometer was used to measure the absorbance of the supernatant at 232 nm, indicating the amount of drug not encapsulated. The entrapment efficiency (EE) was calculated by subtracting the quantity of free drug from the initial amount of drug, using the following equation:

$$\% \textbf{ Entrapment efficiency} = \frac{\textbf{Initial Concentration} - \textbf{Final Concentration}}{\textbf{Initial Drug Concentration}}$$

Evaluation Freeze Dried Polymeric Micelle of Optimized Formulation

Micromeritic properties: Tests such as angle of repose, bulk density, tapped density, compressibility index, and Hausner's ratio are conducted to evaluate the micromeritic properties of the optimized formulation.

Comparative in vitro drug release profile of pure drug and Polymeric Micelle F4 formulation

The drug release study of both the pure drug and polymeric micelle formulations was conducted using a USP Type II apparatus with 900 mL of pH 6.8 phosphate buffer at 37±0.5°C. The paddle rotation speed was set at 50 rpm. Mesalamine-loaded polymeric micelles (equivalent to 250 mg of Mesalamine) and pure Mesalamine (250 mg) loaded capsules were placed in the dissolution apparatus with a hydrogel plug. At predetermined intervals of 1 hour over a total duration of 7 hours, a 5 mL aliquot of the sample was withdrawn, filtered, and analyzed for the percentage of drug released using UV spectroscopy.

Preparation of polymer plugs

Swelling Index of Polymers: Ten grams of various polymers were weighed, and their initial weights (W_0) were recorded. Each polymer was then placed in a 100 mL measuring cylinder containing 100 mL of phosphate buffer pH 7.4. After soaking for 12 hours, excess phosphate buffer pH 7.4 was carefully removed, and the swollen films were re-weighed (Wt.). This experiment was conducted in triplicate. The percentage swelling was calculated using the following formula:

$$\% \textbf{ SwellingIndex} = \frac{\textbf{Wt.} - \textbf{W}_0}{\textbf{W}_0} * \mathbf{100}$$

Where, Wt. = Weight of swollen matrix at time t;

W_0 = Initial weight of matrix.

Lag Time: The lag time refers to the duration before the plug is expelled from the capsule body, initiating drug release. Lag time was visually assessed using buffer solutions at pH 1.2 and 7.4. A USP paddle apparatus was employed for lag time determination. Capsules were secured to the paddle using cotton thread, and the temperature was maintained at 37°C with a rotation speed of 50 rpm.

Preparation of water insoluble hard gelatin cap body and enteric coated cap of hard gelatin capsule

Preparation of water-insoluble hard gelatin cap body: The specified quantity of ethyl cellulose was dissolved in acetone (at a 1:10 ratio). The gelatin capsule body was immersed in this solution, withdrawn, dried, and then immersed again. This coating process was repeated until the weight increased by 50%.

Preparation of enteric coated cap of hard gelatin capsule: The orodispersible solution consisted of Eudragit S100 dissolved in acetone (12%). The top of a gelatin capsule was immersed in this solution, withdrawn, rinsed, and coated again. This coating process was repeated until the weight increased by 20%.

Development of Pulsincap dosage form of Mesalamine

Water-insoluble ethyl cellulose and Eudragit S100 were meticulously applied to coat both the capsule body and cap. The dip coating method was used for this purpose, aimed at regulating gastric emptying. The coating process was repeated until the capsule body achieved a 50% increase in weight and the cap a 20% increase. Polymeric micelles containing 250 mg of Mesalamine were carefully inserted into the ethyl cellulose-treated capsule bodies. The openings of these capsules, now containing polymeric micelles, were sealed by filling them with specified polymers such as HPMC K100M and HPMC K4M. Additionally, a 5% ethanolic ethyl cellulose solution was used to seal the joints between the capsule body and cap.

Table 2: Formulation table for optimized batch of Polymeric Micelle

Sr. No.	Name of the Ingredient	Quantity (mg)	Role of Ingredients
1	Mesalamine	250	API (Anti-inflammatory agent)
2	Soluplus	200	Polymer (Aids in solubilization of poorly water-soluble drug)
3	Pluronic F-127	200	Polymer (Aids in solubilization of poorly water-soluble drug)

Table 3: Composition of Pulsincap Drug Delivery System of Mesalamine incorporated polymeric micelle

Sr. No.	Formulation	Quantity of prepared Polymeric Micelle (mg)	Hydrogel plug forming polymer (mg)	Quantity of Hydrogel plug forming polymer (mg)	Quantity of treated capsule body (mg)	Quantity of treated cap (mg)	Total weight of Capsule (mg)
1	Optimized formulation(F4)	650	HPMC K 100 M	50	103	66	869
2				70			889
3			HPMC K4M	50			869
4				70			889

Weight Variation Test: The variability in tablet weights reliably reflects the variation in drug content. The average tablet weight was determined by individually weighing 20 tablets using a digital analytical balance.

Dissolution Studies: The pulsatile release formulations were evaluated for dissolution in three different dissolution media: pH 1.2, pH 7.4, and pH 6.8, over durations of two, three, and eight hours, respectively. The dissolution testing apparatus used was the USP Type I dissolution apparatus (basket type). Initially, 900 mL of 0.1 N HCl at pH 1.2 was used for the first 2 hours, followed by 900 mL of pH 7.4 buffer solution for the subsequent 3 hours, and finally, 900 mL of pH 6.8 buffer solution for the remaining 8 hours. The rotation speed of the basket in the dissolution media was maintained at 50 rpm, and the temperature was kept constant at 37.5°C. To maintain sink conditions, 5 mL aliquots of the dissolution media were withdrawn at specified intervals and replaced with fresh media. The withdrawn samples were analyzed for drug release at 232 nm using UV spectroscopy in pH 1.2, 7.4, and 6.8 buffers. The average cumulative percentage drug release over the defined time points was calculated.

Stability studies: The appearance, shell integrity, entrapment efficiency, and percentage of drug release of the optimized formulation were assessed after storage at room temperature according to ICH guidelines. The formulation was sealed in a container and stored at room temperature. After a three-month period, the contents were evaluated and examined for any changes in appearance, shell integrity, entrapment efficiency, percentage of drug release, and other parameters.

RESULT AND DISCUSSION

Preformulation Studies

Organoleptic Properties: Upon visual inspection, the drug appeared as a tan to pinkish powder with a slight characteristic odor.

Determination of Melting Point: The melting point of Mesalamine was determined using the capillary method with a Thiel's tube, and it was found to be 280°C.

Determination of Solubility: Mesalamine demonstrated solubility in hydrochloric acid and phosphate buffers, and was slightly soluble in distilled water, acetone, and ethanol. It exhibited very slight solubility in chloroform and ether.

Table 4: Solubility of Mesalamine in different solvents

Sr. No.	Solvents	Solubility (mg/ml)
1.	Distilled Water	0.8 mg/ml
2.	Acetone	3.4 mg/ml
3.	0.1N HCL (PH 1.2)	5.8 mg/ml
4.	Phosphate Buffer (pH 7.4)	3.8 mg/ml
5.	Phosphate Buffer (pH 6.8)	3.5 mg/ml

Determination of λmax by using UV – Spectroscopy

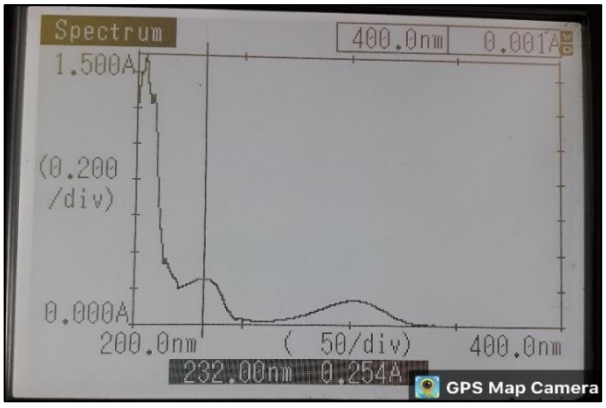


Figure 1: Lambda max of Mesalamine

The maximum absorption wavelength of Mesalamine was determined to be 232 nm in phosphate buffer pH 6.8, consistent with the literature value. This wavelength was selected for further detailed study.

Calibration curve of Mesalamine

0.1 N Hydrochloric Acid (pH 1.2) The absorbance data of the standard solutions is shown in Table 5.

Table 5: Calibration curve of Mesalamine in 0.1 N Hydrochloric Acid (pH 1.2)

Conc.(µg/ml)	Abs.(nm) in pH 1.2	Abs.(nm) in pH 7.4	Abs.(nm) in pH 6.8
2	0.188	0.178	0.168
4	0.245	0.234	0.258
6	0.353	0.335	0.332
8	0.433	0.425	0.487
10	0.589	0.549	0.638

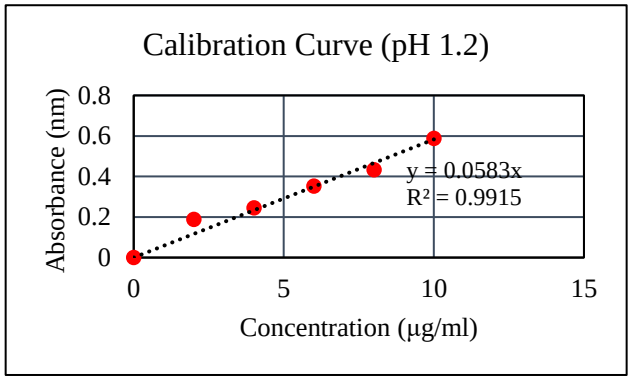


Figure 2: Calibration Curve (pH 1.2)

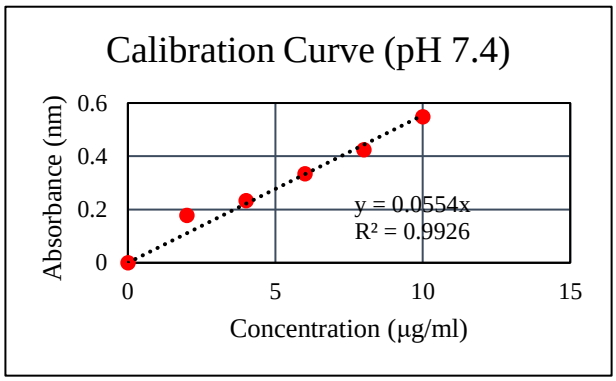


Figure 3: Calibration Curve (pH 7.4)

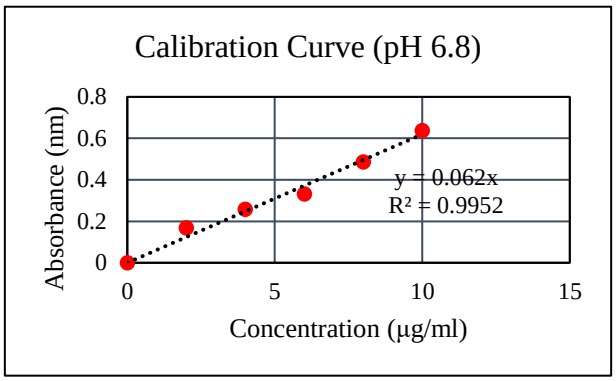


Figure 4: Calibration Curve (pH 6.8)

Drug-Excipients Compatibility Studies

Fourier Transformed Infrared (FT-IR) Spectroscopic Analysis: The FTIR spectra of Mesalamine and other excipients were analyzed to identify characteristic peaks using an FTIR spectrophotometer. In this study, compatibility was assessed using infrared spectroscopy (IR) to determine potential chemical interactions between the pure drug Mesalamine and its physical mixture with excipients.

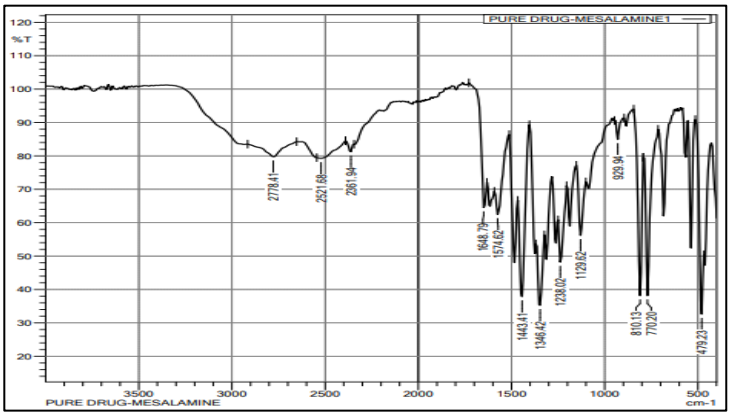


Figure 5: FTIR spectra of pure drug (Mesalamine)

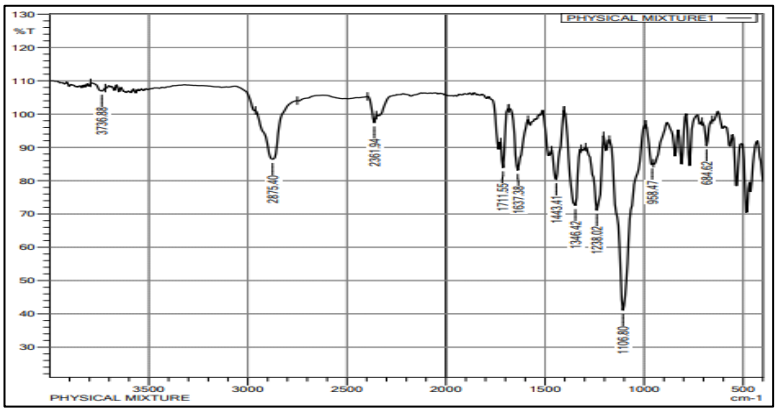


Figure 6: FTIR spectra of Physical Mixture

Differential Scanning Calorimetry: Mesalamine's DSC thermogram determines a conspicuous, abrupt endothermic peak at 281.1°C. The DSC thermogram of a physical combination of Mesalamine, Pluronic F127, Soluplus, and HPMC K100M divulged a conspicuous endothermic peak at 280.8° C.

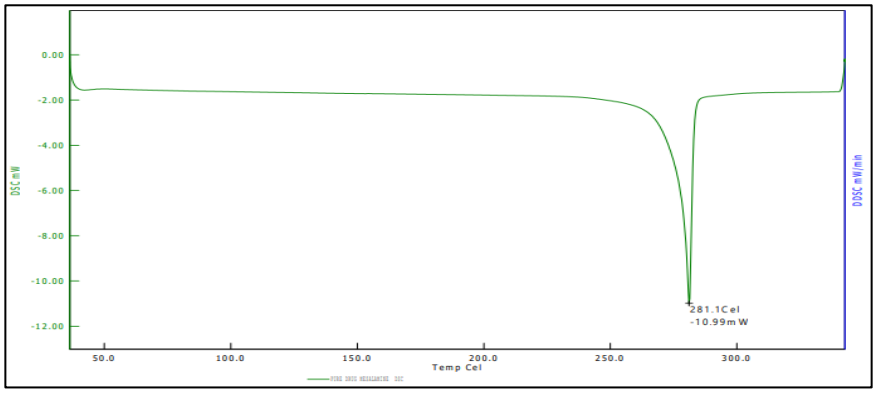


Figure7: DSC Thermogram of Mesalamine

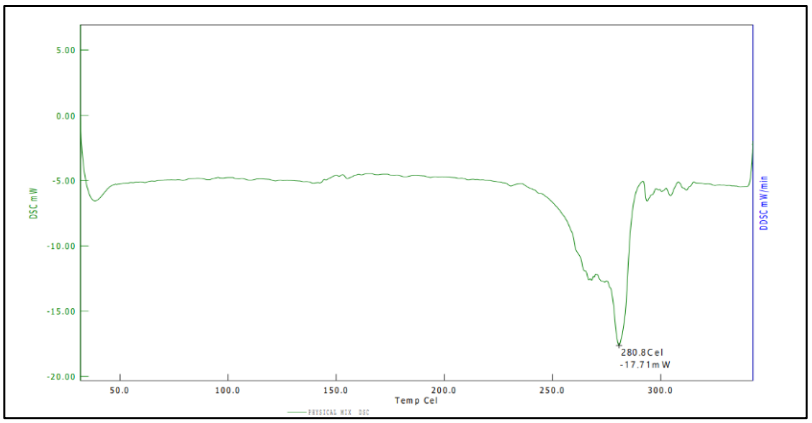


Figure 8: DSC Thermogram of Physical Mixture

Formulation Studies

Determination of Critical Micelle Concentration (CMC)

The critical micelle concentration (CMC) was determined by plotting absorbance against the logarithm of concentrations. Different combinations of selected polymers were used to formulate polymeric micelles, with nine combinations tested to determine the CMC for each formulation. The CMC values for Pluronic F127 and Soluplus were found to be 0.004% w/v and 7×10^{-4}% w/v, respectively. A significant reduction in CMC value was observed with the 2:2 ratio of Soluplus and Pluronic F127. According to literature, a lower CMC value enhances the structural integrity and stability of mixed micelles upon dilution.

Table 6: Determination of Critical Micelle Concentration (CMC)

Sr. No.	Polymer Ratio		Critical Micelle Concentration (% w/v)
	Soluplus (mg)	Pluronic F-127 (mg)	
1.	200	100	2.5×10^{-3}
2.	300	100	7.5×10^{-3}
3.	400	100	6×10^{-3}
4.	200	200	1.5×10^{-3}
5.	300	200	5×10^{-2}
6.	400	200	3×10^{-3}
7.	200	300	1.5×10^{-2}

8.	300	300	2.5×10^{-2}
9.	400	300	2×10^{-2}

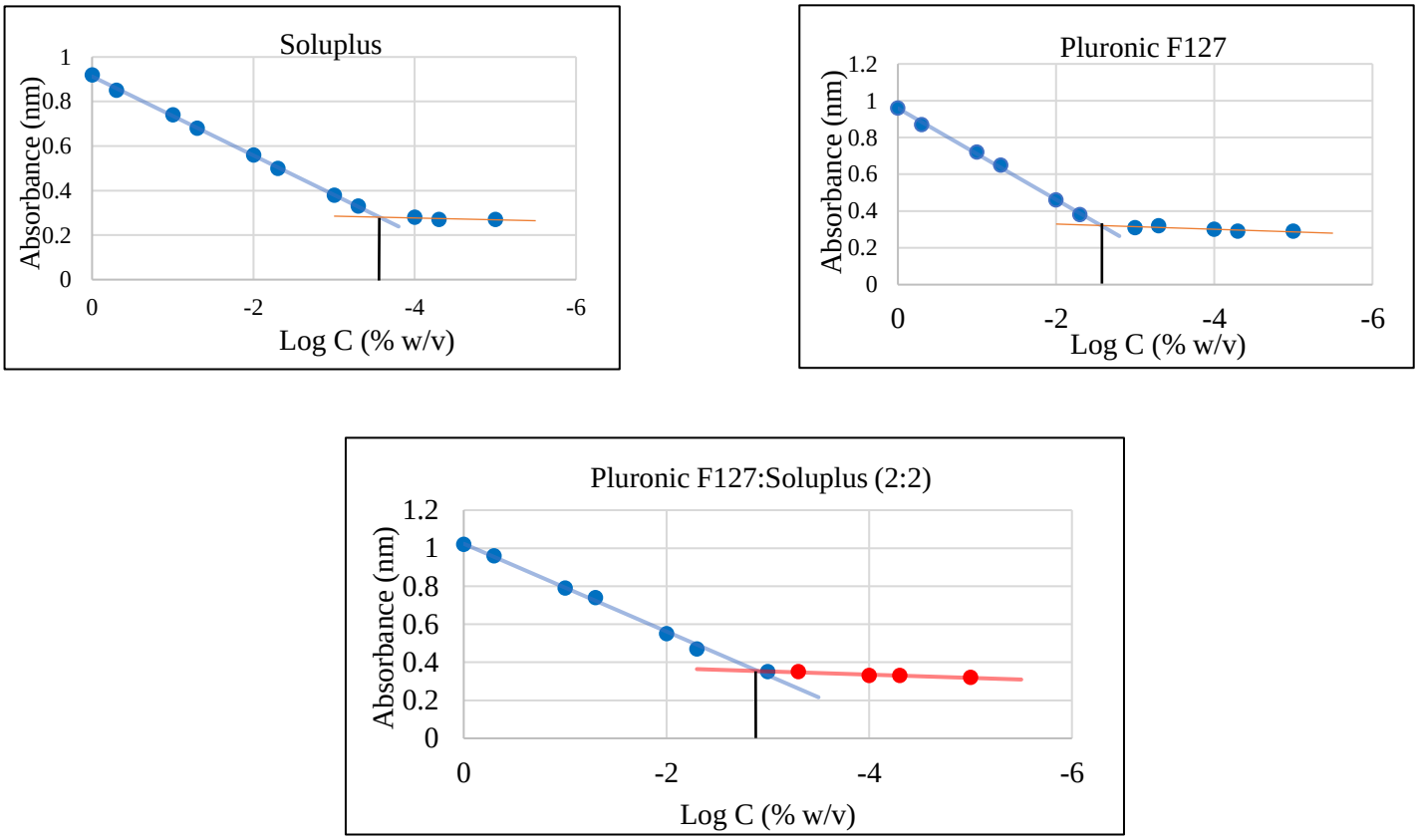


Figure 9: Determination of Critical Micelle Concentration

Characterization of Polymeric Micelle Solution

Measurement of particle size and polydispersity index was conducted on the dispersed formulations after dilution (1:100). Specifically, 1 mL of formulation was dispersed in 100 mL of distilled water to achieve an appropriate count rate for accurate measurement.

Table 7: Particle Size and Polydispersity Index

Sr. no.	**Formulation**	**Particlesize(nm)**	**PDI**
1.	F1	85.2	0.672
2.	F2	72.5	0.550
3.	F3	69.7	0.498
4.	F4	59.7	0.138
5.	F5	63.5	0.376
6.	F6	65.1	0.485
7.	F7	89.1	0.791
8.	F8	95.7	0.829
9.	F9	82.5	0.658
10.	Blank Micelle	54.7	0.126

The particle sizes of polymeric micelle solutions ranged from 59.7 nm to 95.7 nm, with the blank micelle measuring 54.7 nm. The polydispersity index (PDI) for polymeric micelle solutions ranged from 0.138 to 0.829, whereas the blank micelle had a PDI of 0.126. Formulation F4 exhibited the smallest particle size at 59.7 nm, with a polydispersity index

of 0.138. The low polydispersity index (0.138) observed in formulation F4 indicates a uniform distribution of particles throughout the formulation.

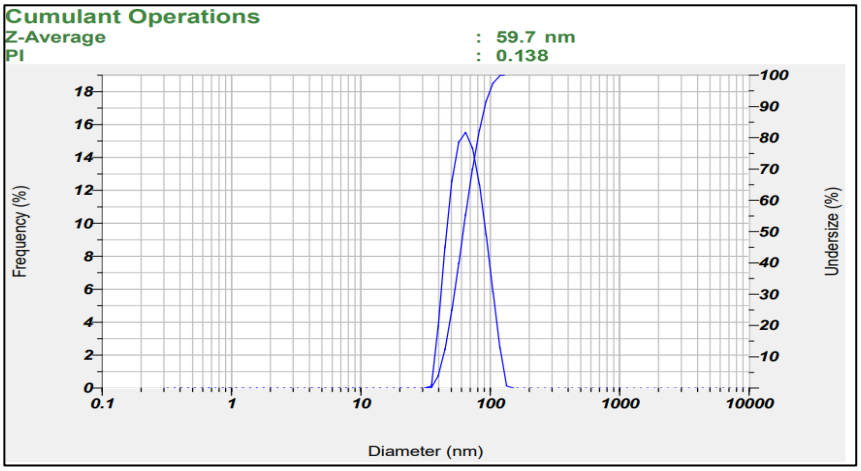


Figure 10: Particle size and Polydispersity Index of Formulation F4

Zeta Potential Measurement

The zeta potential results of various formulations are presented in Table 8and Figure11. The zeta potential of polymeric micelle solutions ranged from -11.9 mV to -32.1 mV, while that of the blank micelle was -31.5 mV. Formulation F4 exhibited a stable zeta potential value of approximately -32.1 mV. The negative zeta potential values indicate formulation stability, hence formulation F4 was selected as the optimized formulation. These results are summarized in Table 8.

Entrapment efficiency was determined by measuring absorbance using a UV spectrophotometer, with results ranging from 39.16% to 87.95%. These findings are presented in Table 8, where formulation F4 demonstrated the highest entrapment efficiency at 87.95% compared to other formulations.

Table 8: Zeta Potential Measurement

Sr. no.	Formulation	Zeta Potential (mV)	Entrapment Efficiency (%)
1.	F1	-18.4	73.95
2.	F2	-21.9	62.79
3.	F3	-24.6	67.12
4.	F4	-32.1	87.95
5.	F5	-19.5	75.34
6.	F6	-16.3	60.90
7.	F7	-12.1	54.89
8.	F8	-13.5	46.90
9.	F9	-11.9	39.16
10.	Blank Micelle	-31.5	-

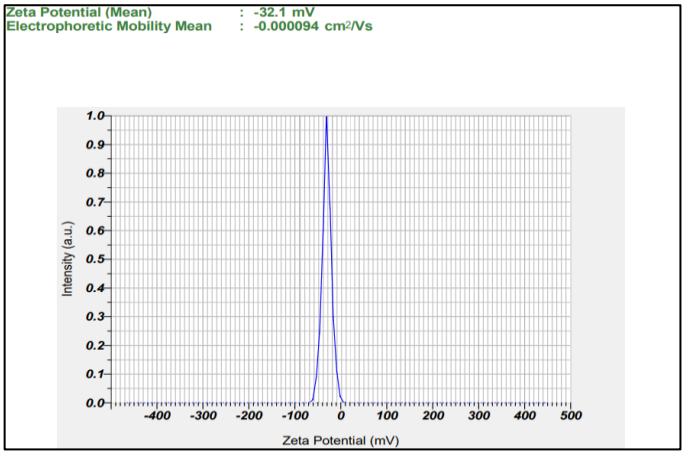


Figure 11: Zeta Potential of optimized Formulation F4

Optimization of Polymeric Micelle Solution

The optimized formulation, F4, was selected based on specific criteria: particle size measured at 59.7 nm, entrapment efficiency of 87.95%, polydispersity index of 0.138, and a zeta potential of -32.1 mV, all of which met pharmacopeial specifications.

Characterization of Freeze-Dried Polymeric Micelles

A. Measurement of Particle Size and Polydispersity Index

The dispersed formulations were analyzed following a dilution of 1:100, where 1 mL of formulation was dispersed in 100 mL of distilled water to achieve the necessary count rate for accurate measurement.

Table 9: Particle Size and Polydispersity Index.

Sr. no.	Formulation	Particlesize(nm)	PDI
1.	**F4**	61.5	0.177

The results for particle size and polydispersity index of the optimized formulations are presented in Table 9 and Figure 11. The freeze-dried polymeric micelles exhibited a particle size of 61.5 nm and a polydispersity index of 0.177. The polydispersity index value of 0.177 observed in formulation F4 indicates a uniform distribution of particles throughout the formulation.

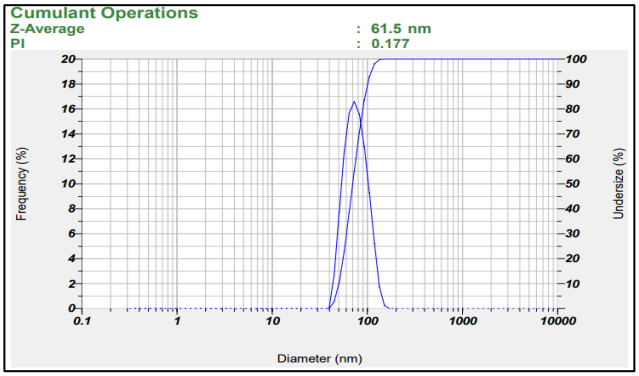


Fig 11: Particle size and Polydispersity Index of Formulation F4

B. Zeta Potential Measurement

The zeta potential magnitude provides insight into the stability of a colloidal system. For the optimized freeze-dried formulation F4, the zeta potential was measured at -36.6 mV, indicating its stability due to the negative zeta potential value. These results are depicted in Table 10 and Figure 12.

Table 10: Zeta Potential Measurement

Sr. no.	Formulation	Zeta Potential (mV)
1.	**F4**	-36.6

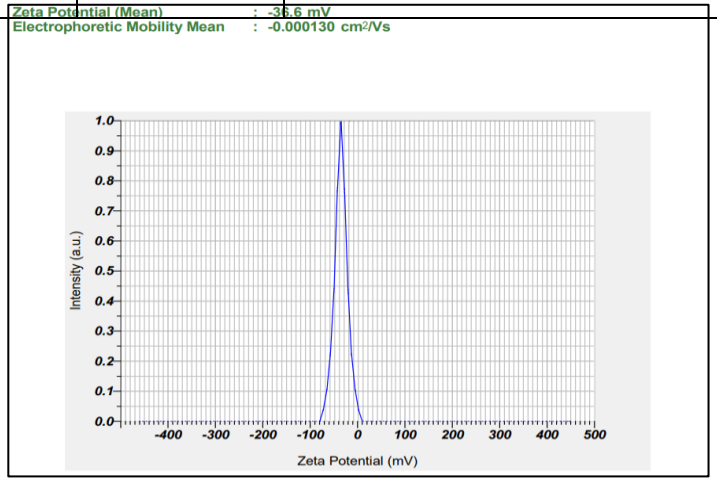


Fig 12: Zeta Potential of Optimized Formulation

C. Determination of Entrapment Efficiency

Entrapment efficiency was determined by measuring the absorbance in UV spectrophotometer. The optimized formulation F4 showed entrapment efficiency about 89.27%. The results are represented in Table 11.

Table 11: Determination of Entrapment Efficiency

Sr. no.	**Formulation**	**Entrapment Efficiency (%)**
1.	**F4**	89.27

The freeze-dried optimized polymeric micelle formulation was assessed for particle size, entrapment efficiency, and zeta potential. Based on these evaluations, formulation F4 exhibited a particle size of 61.5 nm, a polydispersity index of 0.177, a stable zeta potential of -36.6 mV, and an entrapment efficiency of approximately 89.27%.

Assessment of Freeze-Dried Polymeric Micelles in Optimized Formulation F4

A. Solubility

Table 12: Solubility

Sr. No.	**Solvent**	**Solubility (mg/ml)**	
		Pure Drug	**Polymeric Micelle**
1.	Distilled Water	0.8 mg/ml	2.8 mg/ml
2.	Buffer (pH 6.8)	3.5 mg/ml	12.7 mg/ml

B. Micromeritic properties

The results of flow property assessments conducted on the blend of the newly formulated Mesalamine-incorporated polymeric micelles are presented in Table 13. The formulation exhibited an angle of repose of 23.03°, indicating excellent flow characteristics of the powder blend. The compressibility index was 8.06%, with a bulk density of 0.57 g/mL and tapped density of 0.62 g/mL, underscoring its favorable compressibility. The Hausner's ratio for the formulation was 1.08, which suggests exceptional flow properties, as Hausner's ratio values up to 1.14 are indicative of excellent flow patterns.

Table 13: Micromeritic properties

Sr. No.	**Parameter**	**Result**
1.	Bulk density (g/ml)	0.57
2.	Tapped density (g/ml)	0.62
3.	Compressibility index (%)	8.06 %
4.	Hausner's ratio	1.08
5.	Angle of repose	23.03°

Drug excipient Compatibility Study

Fourier Transform Infrared Spectroscopy (FTIR) Analysis: All distinctive peaks of Mesalamine were identifiable in the spectra of the Final Formulation. The study confirms that there was no alteration of the chromophore, as the major peaks of Mesalamine remained unchanged in the Final Formulation, indicating their thorough mixing. All characteristic peaks of Mesalamine were observed in the spectrum of the final formulation, suggesting compatibility between the drug and excipients. The overall spectrum indicated that there was no significant shift in the chemical integrity of the pure drug. According to the data, there was no chemical mismatch between the drug and the excipients used in the formulation

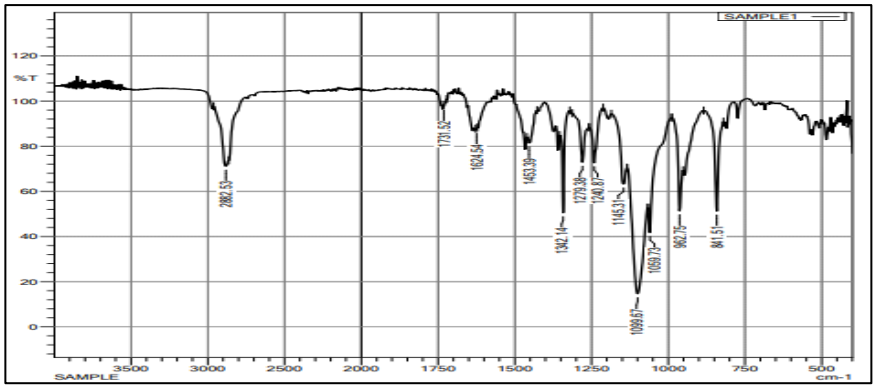


Fig 13: FTIR Spectrum of Optimized Formulation of polymeric micelle

2. Differential Scanning Colorimetric (DSC) Studies

The Mesalamine-incorporated polymeric micelle-filled Pulsincap was analyzed using Differential Scanning Calorimetry (DSC), and the DSC curve is depicted in Figure 14. The DSC thermogram of the prepared polymeric micelle for formulation F4 exhibited a characteristic endothermic peak at 267.5°C. The DSC analysis of Mesalamine and the final formulation indicated that the melting point of Mesalamine underwent negligible change upon combination with other additives, suggesting no alteration or interaction between the drug and excipients.

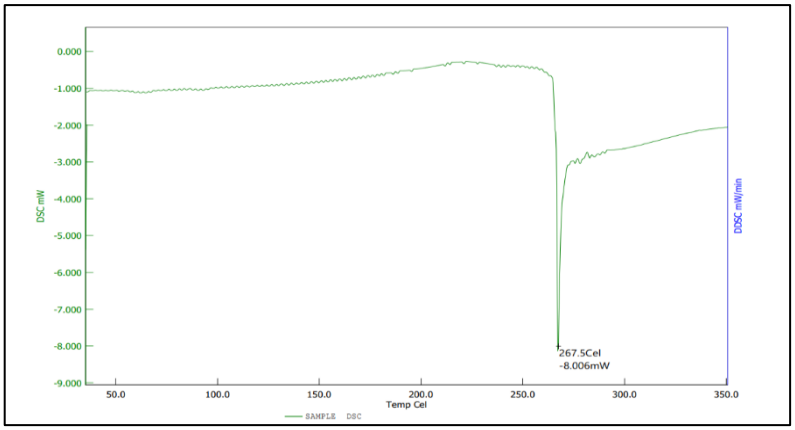
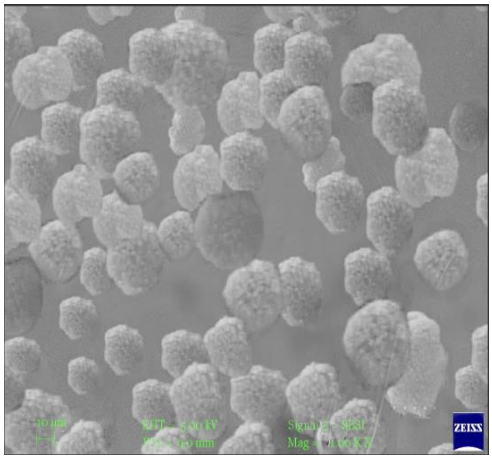


Fig. 14: DSC Thermogram of Final Formulation

D. Morphological analysis of Polymeric Micelle

Scanning Electron Microscopy (SEM) of Polymeric Micelles: SEM micrographs of blank polymeric micelles and Mesalamine-loaded micelles were utilized to examine the morphological characteristics of the prepared polymeric micelles. Both SEM images of blank polymeric micelles and drug-loaded polymeric micelles indicate that the particles are spherical and well separated. Additionally, the images reveal uniform distribution of particles (see Fig. 15 and Fig. 16).

The increased size observed in the drug-loaded polymeric micelles compared to the blank micelles suggests that the incorporation of the drug into the polymeric micelles led to an increase in particle size (see Fig. 15 and Fig. 16).



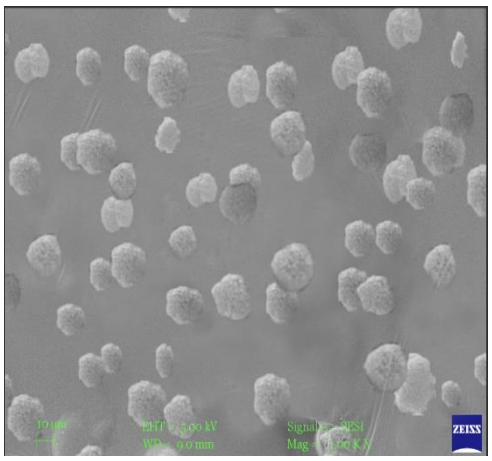


Figure 15: Blank polymeric micelle

Figure 16: Mesalamine loaded polymeric micelle

Comparative in vitro release profile of pure drug and drug loaded polymeric micelle of F4

The optimized formulation exhibited a drug release percentage of 88.63% after 7 hours. In contrast, the drug release of pure Mesalamine was 38.26% after 5 hours. Therefore, the drug release from the polymeric micelle formulation significantly improved. Given that Mesalamine is a BCS Class IV drug, enhancing its solubility can potentially increase its bioavailability.

Table 14: % Cumulative Drug Release of Pure Drug and Optimized Polymeric Micelle F4 Formulation

Sr. No.	Time in Hours	Percent Drug Release of Pure Drug	Percent drug release of F4
1.	1	18.6	30.92
2.	2	24.04	41.39
3.	3	26.87	53.1
4.	4	32.08	70.27
5.	5	38.26	75.68
6.	6	37.86	85.33
7.	7	37.28	88.63

Preparation of polymeric plug

The swelling index was employed to select the polymer plugs used to seal the perforations of capsule shells. These included Hydroxyethyl cellulose, Hydroxypropyl methylcellulose K100M, Hydroxypropyl methylcellulose K4M, Hydroxypropyl methylcellulose E50, Microcrystalline cellulose (MCC), Sodium alginate, Carbopol 934, Hydroxypropyl methylcellulose 15 cp, and Sodium carboxymethyl cellulose (sodium CMC).

Swelling Index: The swelling indices of hydrogel-forming polymers in Pulsincap were assessed. The swelling indices of various polymers are presented in Table 15. Based on the results, Hydroxypropyl Methylcellulose (HPMC) K100M and HPMC K4M exhibited the highest swelling indices and were consequently chosen for the preparation of the polymer plug.

Table 15: Swelling Index

Time	HPMC	HPMC K100M	HPMC K4M	HPMC 15CP	HPMC E50	Sodium CMC	Carbopol 934	Sodium Alginate	MCC	Hydroxy Ethyl Cellulose
30 min	30	38	36	22	34	22	24	20	25	30
1 hr.	37	48	45	26	45	24	29	23	29	40
90 min	42	52	50	35	52	27	36	26	31	48

2 hrs.	49	58	54	44	56	30	42	28	33	60
4 hrs.	55	70	68	46	67	36	49	38	48	66
6 hrs.	67	74	70	53	71	40	66	43	65	72
8 hrs.	72	82	79	57	77	47	72	50	70	78
10 hrs.	78	87	82	62	80	49	80	60	75	82
12 hrs.	80	89	87	65	83	50	82	62	76	84

Physicochemical properties of Hydrogel Plug forming Polymers (Lag time)

The hydrogel-forming polymers were assessed for their physicochemical properties, as detailed in Table 16. In the optimized formulation F4, the polymer plug took 300 minutes to achieve optimal swelling and ejection from the capsule body. Additionally, the optimal lag time was determined to be between 5 to 6 hours.

Table 16: Physicochemical properties of plug

Sr. NO.	Formulation Code	Polymer Name	Wight in mg	Lag time in min
1.	F4	HPMC K4M	50	4 hours 40 min
2.	F4	HPMC K4M	70	4 hours 49 min
3.	F4	HPMC K100M	50	4 hours 50 min
4.	F4	HPMC K100M	70	5 hours

Development of Pulsincap dosage form of Optimized Formulation F4 containing Mesalamine

The capsule body and cap were fully coated with water-insoluble ethyl cellulose and Eudragit S100, respectively. Polymeric micelles containing 250 mg of mesalamine were manually filled into the ethyl cellulose-treated capsule bodies. Subsequently, the openings of the capsule bodies containing polymeric micelles were closed by plugging them with the selected polymer HPMC K100M. Additionally, a small amount of 5% ethanolic solution of ethyl cellulose was applied to seal the joint between the capsule body and cap.

Weight variation test: The weight variation for the Mesalamine incorporated Polymeric micelle containing pulsin cap was found to be 5%.

In Vitro drug release study: The % cumulative drug release from the polymeric micelle formulation reached 91.63% after 12 hours, whereas the drug release from the pure drug was 39.26% after 10 hours. The in vitro drug release from the Pulsincap drug delivery system extended over a period of 13 hours. It was observed that the enteric coating of Eudragit S100 remained intact for 2 hours in pH 1.2 in 0.1 N HCl. Subsequently, the enteric-coated cap of the Pulsincap dissolved in phosphate buffer pH 7.4, allowing the polymer plug to absorb the surrounding fluid, swell, and release a small amount of drug through the swollen matrix. Once fully swollen, the plug ejected from the capsule body, releasing the drug into the colonic fluid of phosphate buffer pH 6.8. The prepared enteric-coated Pulsincap formulation met the lag time requirement of 5 hours, aligning with colon targeting and treatment site criteria.

Table 17: % Cumulative Drug Release of Pure Drug and polymeric micelle F4 loaded Pulsincap

		% Cumulative Drug Release	
	Time (hrs.)	**Pure Drug**	**Polymeric micelle**
pH 1.2 0.1 N HCL	0	0	0
	1	0	0
	2	0	0
pH 7.4 Buffer Solution	3	5.27	6.33
	4	6.15	7.89
	5	8.84	8.75
pH 6.8 Buffer Solution	6	19.6	32.92
	7	25.04	44.39
	8	27.87	56.1
	9	33.08	73.27
	10	39.26	79.68

	11	38.86	87.33
	12	38.28	91.63
	13	37.51	90.38

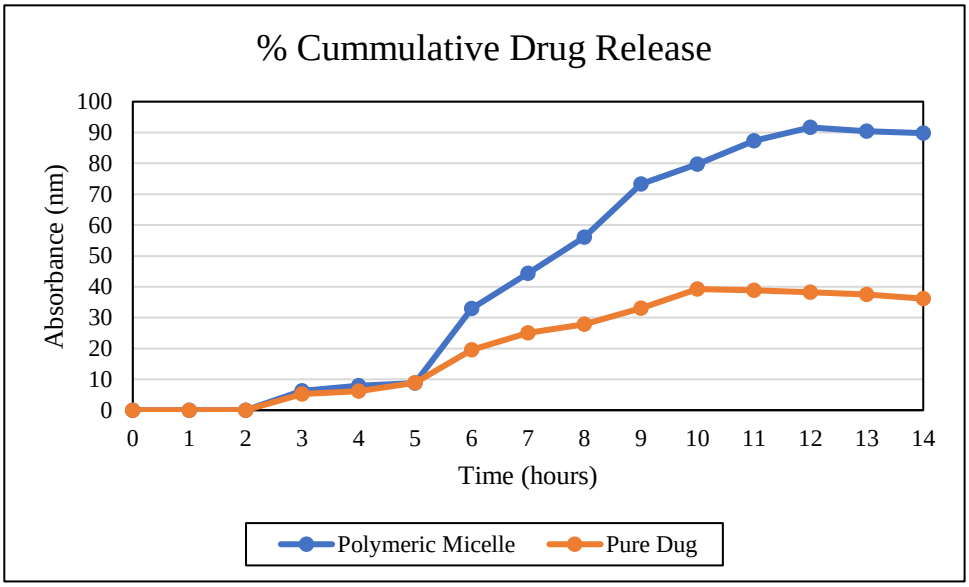


Figure 17: Comparative in vitro release study of pure drug and polymeric micelle loaded Pulsincap.

Stability Study

Based on the evaluation, there were no significant changes observed in the appearance, shell integrity, entrapment efficiency, and % drug release of the Mesalamine-incorporated polymeric micelle filled Pulsincap. Even after 3 months, the formulation released 89.70% of the drug after 12 hours. A slight change in drug release compared to earlier data for formulation F4 was noted, as depicted in Table 18.

Table 18: Stability Study

Sr No.	Period (days)	Appearance	Shell Integrity	Entrapment Efficiency (%EE)	% CDR
1.	0 Days	No Change	No damage	87.94 %	91.63 %
2.	30 Days	No Change	No damage	87.40 %	90.84 %
3.	60 Days	No Change	No damage	86.94 %	90.41 %
4.	90 Days	No Change	No Damage	86.63%	89.70%

CONCLUSION

Mesalamine, classified under 5-Aminosalicylate, is a primary treatment for IBD, hence selected for this study. Soluplus and Pluronic F-127 were chosen for their ability to enhance solubility of poorly water-soluble drugs. Among various ratios tested (2:1, 3:1, 4:1, 2:2, 3:2, 4:2, 2:3, 3:3, 4:3), the 2:2 ratio exhibited the lowest CMC, indicating stable micelle formation. Polymeric micelle formulations were prepared via solvent evaporation with fixed drug and varying Soluplus and Pluronic F-127 amounts. Formulation F4 emerged as optimal, showing smallest particle size, low polydispersity, stable zeta potential, and highest entrapment efficiency.

Formulation F4 was freeze-dried to obtain polymeric micelle powder with particle size of 61.5 nm, entrapment efficiency of 89%, polydispersity index of 0.177, and zeta potential of -36.6. SEM analysis confirmed spherical, well-separated particles. Solubility of Mesalamine in polymeric micelle form increased 3.5 times compared to pure drug. F4 exhibited excellent flow properties in terms of Angle of Repose, Bulk Density, Tapped Density, Carr's Compressibility Index, and Hausner's Ratio.

In vitro, Mesalamine-loaded polymeric micelle formulation (F4) achieved 91.63% cumulative drug release after 12 hours, compared to 39.26% for pure drug over 10 hours. The pulsincap device, designed for colon-targeted delivery,

supports local anti-inflammatory action. Stability studies over 90 days confirmed F4's stability with no significant changes in appearance, shell integrity, entrapment efficiency, or drug release profile.
The enteric-coated pulsincap loaded with Mesalamine-loaded polymeric micelle and HPMC K100M hydrogel plug effectively provided a lag time for drug release in the large bowel, supported by Eudragit S100 (12%) in acetone as a mucin carrier.

REFERENCES

1. Burger, D.; Travis, S. Conventional Medical Management of Inflammatory Bowel Disease. Gastroenterology2011, 140 (6), 1827-1837.e2. https://doi.org/10.1053/j.gastro.2011.02.045.
2. Ogura, Y.; Bonen, D. K.; Inohara, N.; Nicolae, D. L.; Chen, F. F.; Ramos, R.; Britton, H.; Moran, T.; Karaliuskas, R.; Duerr, R. H.; Achkar, J. P.; Brant, S. R.; Bayless, T. M.; Kirschner, B. S.; Hanauer, S. B.; Nũez, G.; Cho, J. H. A Frameshift Mutation in NOD2 Associated with Susceptibility to Crohn's Disease. Nature2001, 411 (6837), 603–606. https://doi.org/10.1038/35079114.
3. Podolsky, D. K. Inflammatory Bowel Disease. New England Journal of Medicine2002, 347 (6), 417–429. https://doi.org/10.1056/NEJMRA020831.
4. Halling, M. L.; Kjeldsen, J.; Knudsen, T.; Nielsen, J.; Hansen, L. K. Patients with Inflammatory Bowel Disease Have Increased Risk of Autoimmune and Inflammatory Diseases. World J Gastroenterol2017, 23 (33), 6137–6146. https://doi.org/10.3748/wjg.v23.i33.6137.
5. Hanauer, S. B. Aminosalicylates in Inflammatory Bowel Disease. Aliment Pharmacol Ther2004, 20 (s4), 60–65. https://doi.org/10.1111/J.1365-2036.2004.02048.X.
6. Cai, Q. X.; Zhu, K. J.; Chen, D.; Gao, L. P. Synthesis, Characterization and in Vitro Release of 5-Aminosalicylic Acid and 5-Acetyl Aminosalicylic Acid of Polyanhydride - P(CBFAS). European Journal of Pharmaceutics and Biopharmaceutics2003, 55 (2), 203–208. https://doi.org/10.1016/S0939-6411(02)00191-1.
7. Molodecky, N. A.; Soon, I. S.; Rabi, D. M.; Ghali, W. A.; Ferris, M.; Chernoff, G.; Benchimol, E. I.; Panaccione, R.; Ghosh, S.; Barkema, H. W.; Kaplan, G. G. Increasing Incidence and Prevalence of the Inflammatory Bowel Diseases with Time, Based on Systematic Review. Gastroenterology2012, 142 (1). https://doi.org/10.1053/j.gastro.2011.10.001.
8. Silverberg, M. S.; Satsangi, J.; Ahmad, T.; Arnott, I. D. R.; Bernstein, C. N.; Brant, S. R.; Caprilli, R.; Colombel, J. F.; Gasche, C.; Geboes, K.; Jewell, D. P.; Karban, A.; Loftus, E. V.; Peña, A. S.; Riddell, R. H.; Sachar, D. B.; Schreiber, S.; Steinhart, A. H.; Targan, S. R.; Vermeire, S.; Warren, B. F. Toward an Integrated Clinical, Molecular and Serological Classification of Inflammatory Bowel Disease: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Can J Gastroenterol Hepatol2005, 19, 5A-36A. https://doi.org/10.1155/2005/269076.
9. Klotz, U. The Role of Aminosalicylates at the Beginning of the New Millennium in the Treatment of Chronic Inflammatory Bowel Disease. Eur J Clin Pharmacol2000, 56 (5), 353–362. https://doi.org/10.1007/S002280000163.
10. Fakhoury, M.; Negrulj, R.; Mooranian, A.; Al-Salami, H. Inflammatory Bowel Disease: Clinical Aspects and Treatments. J Inflamm Res2014, 7 (1), 113–120. https://doi.org/10.2147/JIR.S65979.
11. Eliakim, R.; Suissa, A.; Yassin, K.; Katz, D.; Fischer, D. Wireless Capsule Video Endoscopy Compared to Barium Follow-through and Computerised Tomography in Patients with Suspected Crohn's Disease - Final Report. Digestive and Liver Disease2004, 36 (8), 519–522. https://doi.org/10.1016/j.dld.2004.03.011.
12. Vilela, E. G.; da Gama Torres, H. O.; Martin, F. P.; de Lourdes de Abreu Ferrari, M.; Andrade, M. M.; da Cunha, A. S. Evaluation of Inflammatory Activity in Crohn's Disease and Ulcerative Colitis. World J Gastroenterol2012, 18 (9), 872–881. https://doi.org/10.3748/WJG.V18.I9.872.
13. Aminu, N.; Bello, I.; Umar, N. M.; Tanko, N.; Aminu, A.; Audu, M. M. The Influence of Nanoparticulate Drug Delivery Systems in Drug Therapy. Journal of Drug Delivery Science and Technology. Editions de Sante December 1, 2020. https://doi.org/10.1016/j.jddst.2020.101961.
14. Riehemann, K.; Schneider, S. W.; Luger, T. A.; Godin, B.; Ferrari, M.; Fuchs, H. Nanomedicine—Challenge and Perspectives. Angewandte Chemie International Edition2009, 48 (5), 872–897. https://doi.org/10.1002/ANIE.200802585.
15. Ashara, K. C.; Paun, J. S.; Soniwala, M. M.; Chavada, J. R.; Badjatiya, J. K. Nanoparticulate Drug Delivery System: A Novel Approach. International Journal of Drug Regulatory Affairs2018, 1 (2), 39–48. https://doi.org/10.22270/ijdra.v1i2.109.

16. Bamrungsap, S.; Zhao, Z.; Chen, T.; Wang, L.; Li, C.; Fu, T.; Tan, W. Nanotechnology in Therapeutics: A Focus on Nanoparticles as a Drug Delivery System. https://doi.org/10.2217/nnm.12.872012, 7 (8), 1253–1271. https://doi.org/10.2217/NNM.12.87.
17. Fissell, W. H. What Is Nanotechnology? Adv Chronic Kidney Dis2013, 20 (6), 452–453. https://doi.org/10.1053/j.ackd.2013.08.008.
18. Hu, J.; Johnston, K. P.; Williams, R. O. Nanoparticle Engineering Processes for Enhancing the Dissolution Rates of Poorly Water Soluble Drugs. Drug Dev Ind Pharm2004, 30 (3), 233–245. https://doi.org/10.1081/DDC-120030422.
19. Wilczewska, A. Z.; Niemirowicz, K.; Markiewicz, K. H.; Car, H. Nanoparticles as Drug Delivery Systems. Pharmacol Rep2012, 64 (5), 1020–1037. https://doi.org/10.1016/S1734-1140(12)70901-5.
20. Aminu, N.; Toh, S. M. Applicability Of Nanoparticles-Hydrogel Composite In Treating Periodontal Diseases And Beyond. Asian Journal of Pharmaceutical and Clinical Research2017, 10 (2), 65–70. https://doi.org/10.22159/AJPCR.2017.V10I2.15709.
21. Prabahar, K.; Alanazi, Z.; Qushawy, M. Targeted Drug Delivery System: Advantages, Carriers and Strategies. Indian Journal of Pharmaceutical Education and Research2021, 55 (2), 346–353. https://doi.org/10.5530/ijper.55.2.72.
22. Mahajan, H. S.; Patil, S. B.; Gattani, S. G.; Kuchekar, B. S. Targeted Drug Delivery Systems. Pharma Times2007, 39 (2), 19–21.
23. Şengel-Türk, C. T.; Hasçiçek, C.; Gönül, N. Colon Targeted Drug Delivery Systems. Ankara Universitesi Eczacilik FakultesiDergisi2006, 35 (2), 125–148.
24. Bauditz, J.; Wedel, S.; Lochs, H. Thalidomide Reduces Tumour Necrosis Factor Alpha and Interleukin 12 Production in Patients with Chronic Active Crohn's Disease. Gut2002, 50 (2), 196–200. https://doi.org/10.1136/GUT.50.2.196.
25. Riess, G. Micellization of Block Copolymers. Prog Polym Sci2003, 28 (7), 1107–1170. https://doi.org/10.1016/S0079-6700(03)00015-7.